Center Reflections

A monthly publication highlighting activities at the W.M. Keck Foundation Center for Molecular Structure

California State University Fullerton

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Disk Crash, Staff Changes and an Improved Website

CMolS experienced a major disk crash on our SGI server this past summer. This wiped out much of our website and cut off e-mail service to the x-ray laboratory in late July, August and early September. After exchanging bad cables and new disks that had been damaged in shipment, we were able to restore files from the archives. However, our most recent backups were accidentally erased by one of the storage device technical support staff, who placed them on top of a monitor. Crystallographic data, which is archived on CD-ROM, was not affected.

The website has been restored and you will note some changes. The electronic request form for instrument and computer time has been streamlined. Also, all issues of *Center Reflections* are available for download from the site in *.pdf* format.

In July, our staff scientist, Dr. Brian Schick, accepted a staff position in the Macromolecular Crystallography Laboratory at Lawrence Livermore National Laboratory, a position which he assumed on September 1. Brian was a vital member of the laboratory and his presence is deeply missed. We wish him well in his new position. Our last search for his replacement was not successful, and we will open a new search in the next few weeks. If any of our users can recommend someone for this position, forward this information to us as soon as possible.

Growing Crystals that Will Make Your Crystallographer Happy

Paul Boyle, who directs the x-ray facility at the North Carolina State University Department of Chemistry, has written an excellent guide for graduate students and other researchers wanting to grow crystals suitable for x-ray structure determinations. The monograph is meant neither to be rigorous nor exhaustive, but rather, a sort of practical "how to" cookbook. A good general reference which covers similar material can be found in "Crystal Growing", Chemistry in Britain, 1981, 17, 222-225 by Peter G. Jones. The important points, which will help our colleagues to prepare diffraction quality crystals for x-ray analysis, are summarized here. The emphasis is on small molecule crystallization, although the general principles of crystal growth for small molecules and macromolecules are similar. The next article in this issue addresses protein crystallization specifically.

Your goal in growing a single crystal for an x-ray diffraction experiment at CMolS is first and foremost to grow a crystal of suit-

able size. Although you may be tempted to grow a crystal as large as possible or, conversely, to be happy with anything that looks like it might be crystalline, the optimum size is one which has dimensions of 0.2 - 0.4 mm and as uniformly in all dimensions as possible. The perfect crystal would be a sphere. The majority of structure determinations are delayed by a lack of suitable crystals.

A number of factors during crystal growth affect the size and quality of crystals. One must choose a solvent in which the compound of interest has a suitable solubility for recrystallization. The number and nature of nucleation sites is important. The system may require mechanical agitation and time for proper crystal growth to occur.

Your compound should be moderately soluble in the solvent chosen. On the last page of this newsletter is a table of commonly used solvents and some relevant properties. If your compound is too soluble, small crystals will likely form. If it is not very soluble, you will likely end up with an amorphous precipitate. Solvents in which your compound forms a supersaturated solution will tend to yield small crystals.

You will obtain fewer crystals of larger size with fewer nucleation sites. This is desirable. Smaller crystals will result from many nucleation sites. Ambient dust in the laboratory or particulate matter in your solutions will provide lots of nucleation sites in your crystallization vessel. Avoid dust and particulate matter.

Although there have been documented cases where vibration has aided crystallization, as a general rule avoid mechanical disturbance of the crystal growing vessel. This usually results in small crystals. Don't set up crystallization trials next to a vacuum pump, an x-ray generator or a highly trafficked area of the laboratory. Set up crystallization experiments in an area where the temperature is constant. Avoid the temptation to pick up your experiment every day to check on crystals. Crystals need time to grow. Patience is a virtue. If you can resist the temptation to look, leave your experiment alone for several days.

To quote Paul Boyle, "There are as many variations to the basic crystal growing recipes as there are crystallographers." The techniques you use will depend on the chemical properties of the compound of interest. For example, your compound may be air, moisture or temperature sensitive. The best method for growing crystals from compounds that are not sensitive to ambient conditions is by slow evaporation. This involves preparing a saturated or nearly saturated solution of the compound in a suitable solvent. This solution is then transferred to a clean crystal growing vessel and covered, but not airtight. The rate of evaporation may be modulated by covering the vessel with parafilm with holes poked into it, or by changing the surface area of the solution. Beakers, Petrie dishes, watchglasses and test tubes are all viable vessels. Some of the best crystals may be grown in NMR tubes. One can also use mixtures of solvents, with different vapor pressures and in which your compound has variable solubility. Whatever vessel and solvent system you choose, the crystallization experiment should be placed in a quiet place in the laboratory and left to evaporate.

In the case where the solute is less than moderately soluble in the solvent, and the solvent boiling point is less than 100C, one can prepare a saturated solution of the compound in warmed solvent, transfer the solution to a CLEAN large test tube and stopper, then place the test tube in a Dewar flask in which water heated to the same temperature has been added. Stopper the Dewar flask with a cork stopper and let the vessel sit for a week. These techniques can be expanded

to incorporate binary or tertiary solvent systems. One can modulate crystal morphology in this fashion.

Vapor diffusion is a good method for growing crystals from milligram amounts of material. It is discussed in Stout and Jensen's classic text "X-ray Structure Determination" on p. 65.

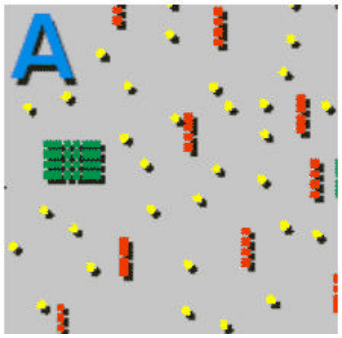
Solvent diffusion is a layering technique also used for milligram amounts of materials that are sensitive to ambient laboratory conditions (air, moisture). Here, you dissolve the solute in solvent 1 and place in a test tube. Then, slowly layer solvent 2 in the test tube. The density of solvent 2 must be less than that of solvent 1. According to Boyle, NMR tubes are excellent vessels to use for this crystal growing technique, and a CH2Cl2/Et_2O solvent combination is a good one to try if your compound is insoluble in ether.

Solutions of the reactants may also be allowed to diffuse into one another. There is mention in the literature of this technique being used with diffusion in silica gels (see Acta Cryst. 19, 483, (1965)).

Protein Crystallization: Techniques and Random Screens

Brent Segelke, LLNL

Protein crystallization is variously referred to as an art (and sometimes a black art), a knack, or a game of chance rather than a science. If one were so bold as to declare protein crystallization a science, it would have to be conceded that it is at least an empirical field. The experiment or condition that yields crystals from a protein of interest cannot be predicted. Instead, select conditions from within the available sample space are tested empirically until a success is discovered. In this regard, crystallization is similar to many other problems that involve screening and optimization, including as games of chance. A probability model can be phenomenologically derived that describes the efficiency of a variety of screening protocols. Examination of this model reveals that the choice of screening protocols depends on the distribution of successes within your sample space. The game of battleship provides a useful analogy.



In the game of battleship, a player has to test locations blindly on a grid upon which an opponent has placed a number of battleships. The player may choose to probe locations on the grid in a systematic block pattern or a more random pattern. There are other choices, but for our purposes we'll just consider the two possibilities. Which sampling pattern is likely to be more successful? If you define success as the fewest number of trials you have to perform on average before finding the first hit on any one battleship, we can use the afore mentioned probability model to help us decide on our preferred sampling pattern. As it turns out, if the whole board is filled with battleships, it doesn't matter which sampling pattern one uses—a predictable result. It also turns out that if battleships each take up only one grid point and they are not placed on adjacent grid points, systematic sampling is still the most successful. In any other case, where individual battleships occupy more than one grid point, and where the whole board is not filled with battleships, random sampling is

more successful than systematic sampling. Generalizing to all similar sampling problems then, if successes are distributed in the sample space such that they are evenly spread amongst the possible trials, a systematic screen is most efficient. If however, successes are clustered together in the sample space, random sampling is inherently more efficient.

So, how are successes distributed in the sample space for protein crystallization? We conducted crystallization trials on five proteins with three sampling protocols popular in macromolecular crystallization. From these experiments it has been determined that successful crystallization experiments are clustered together and that random sampling should be the most efficient screening method.

Random (1) and pseudo-random (2, 3, 4) crystallization screens have been previously described and are increasingly popular techniques for initiating de novo crystallization of macromolecules. There are a number of preformulated random and pseudo-random screens commercially available. We have written a fully customizable, web-based program that generates random screens for you. **CRYSTOOL** (see Websites of Interest column) is a highly efficient random crystallization screen (similar to the Hampton Screens, but with a broader and customizable sampling of parameter space). **CRYSTOOL** is a fully customizable program that will generate any number of random combinations of crystallization conditions from your selections of buffers, precipitants, additives and pH ranges.

Our probability model gives us insight into the preferred sampling method for crystal screening but also gives us some means to determine how thorough we need to been in our search. A cursory review of available literature gives some insight into the probability of finding crystallization conditions, at least for well-behaved proteins. From sixty-six crystallization efforts found in five literature sources, the average proportion of successful experiments for crystallization was approximately 10% and ranged from 2% to 68%. For a protein that has a 2% probability of crystallizing in any one experiment, there is approximately a 36% chance of not finding a useful condition in the 50 trials of the “Sparse Matrix” screen. If one were to employ random sampling and perform 200 randomly generated experiments, the chance of simply failing to find a useful condition is greatly reduced (approximately 2% chance). Having performed these 200 experiments, one can be confident that they are dealing with a stubborn molecule and proceed with more creative techniques to generate cooperative material (such as mutating a large number of hydrophobic side chains).

1. Shieh et. al. 1995
2. Carter et. al. 1979
3. Jancarick et. al. 1991
4. Cudney et. al. 1994
5. Stura, E.A et. al. 1992
6. McPherson, A. 1982
7. Dyda et. al. 1994

Brent Segelke is a post-doctoral crystallographer at Lawrence Livermore National Laboratory. He received his B. S. in Chemistry from the University of California at Davis with an emphasis on physical chemistry, and his Ph.D. in Biochemistry from the University of California at San Diego where he conducted crystallization and structural studies on phospholipase A2 and CD1. After completing a post-doctoral fellowship at The Scripps Research Institute, Brent joined the Macromolecular Crystallography Group at LLNL, where is currently engaged in the

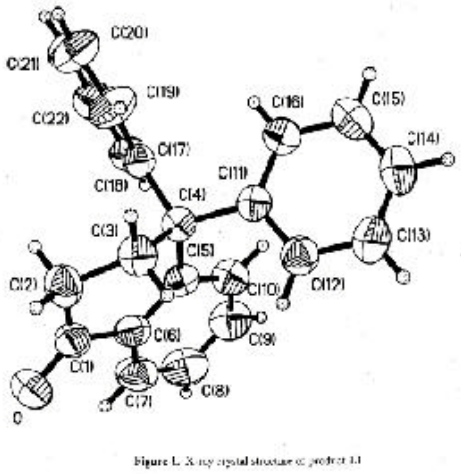
structure determination of molecules involved in human disease and examining the efficiency of sampling protocols, particularly stochastic sampling (**CRYSTOOL**).

Electrophilic Chemistry of Biologically Important a-Ketoacids

CSU Pomona

The research of Professor Douglas Klumpp at Cal Poly Pomona involves the chemistry of dicationic electrophiles generated in the Bronsted superacid, CF_3SO_3H (triflic acid). Dicationic electrophiles are proposed as intermediates in the superacid-catalyzed reactions of 1,2-dicarbonyol compounds, 1,2,3-tricarbonyl compound, heterocyclic ketones or aldehydes, and other compounds. By exploiting the reactivity of dicationic systems, Klumpp's group has prepared a variety of aryl-substituted products in good to excellent yield.

The α-ketoacids and their deprotonated anions play central roles in a number of biochemical processes. Despite their importance, little work has been done to evaluate these compounds and their reactivity towards weak nucleophiles such as aromatic compounds. Given the general reactivity of 1,2-dicarbonyl groups in superacids, it seemed plausible that the α-ketoacids might also display superelectrophilic reactivity. In a recent publication in the ***Journal of Organic Chemistry***[1], Klumpp's laboratory reports studies of the electrophilic chemistry of several of pyruvic acid, α-ketosuccinic acid, α-ketoglutaric acid and phenylpyruvic acid and propose mechanisms for superelectrophilic activation. In particular, when α-ketoglutaric acid is reacted with C_6H_6 in triflic acid, a tetralone derivative is formed in 89% yield as the only major product. The crystal structure of this product, whose ORTEP is shown, was determined at CMolS. Its elucidation by x-ray diffraction was key to establishing the proposed reaction mechanism.



Chen Liu, a senior research student of Kantardjieff's at CMolS in the spring of 1999, solved the structures of two additional products from electrophilic aromatic substitution reactions, whose stereochemistry is indistinguishable by NMR methods. The crystal structures of these compounds, whose formulas are $C_{28}H_{18}O$ and $C_{23}H_{16}O_3$, were by no means trivial to solve and refine, owing to the number of atoms present and difficulties in producing good crystals. To produce crystals suitable for structure determination by x-ray diffraction, Chen employed eighteen different recrystallization schemes, in which she varied thermodynamic parameters such as solvent dielectric constant, temperature, solvent composition and rate of equilibration. These structures will be reported in future publications.

1. Douglas A. Klumpp, Siufu Lau, Manuel Garza, Brian Schick and Katherine Kantardjieff. "The electrophilic chemistry of biologically important α-ketoacids." J. Org. Chem. 19: ASAP (1999).

Douglas Klumpp is Assistant Professor of Chemistry at Cal Poly Pomona. He received his B.S. in Chemistry from the University of Oklahoma and his Ph.D. from Iowa State University. Prior to joining the faculty at Pomona, he was a post-doctoral fellow in the laboratory of George Olah at the University of Southern California. When he is not working in his laboratory, Doug creates interesting paintings with a chemistry theme.

Websites of Interest

- ***Journal of the Chemical Computing Group, Inc.*** http://www.chemcomp.com/ Chemical Computing Group Inc. develops and markets high-end scientific software and services for High Throughput Screening and Computer Aided Molecular Design applied to Life and Materials Sciences.
- ***CRYSTOOL*** http://www-structure.llnl.gov/crystool/crystool.htm *See this issue.*
- ***Growing Crystals that Will Make Your Crystallographer Happy*** http://rocket.chem.ualberta.ca/xray/links.html *See this issue.*
- ***Crystallography 101*** (www-structure.llnl.gov/Xray/101index.html) - see Volume 1, Issue 3 of *Center Reflections*.

Upcoming Events

November 20, 1999: **Southern California Council on Undergraduate Research Annual Conference,** Loyola Marymount University, Los Angeles, CA.

January 13-14, 2000: **12th Annual CSU Biotechnology Symposium,** CalPoly Pomona Kellogg Conference Center.

February 12-16, 2000: **Biophysical Society** Annual Meeting, New Orleans, LA. http://www.biophysics.org/biophys/society/annmtg/

March 26-30, 2000: **American Chemical Society** National Meeting, San Francisco, CA. http://www.acs.org/meetings/sanfran2000/

April 15-18, 2000: **Experimental Biology 2000 FASEB** Meeting and Scientific Exposition, San Diego, CA. http://www.faseb.org/eb2000

July 22-27, 2000: **American Crystallographic Society** Annual Meeting, St. Paul, MN. http://nexus.hwi.buffalo.edu/ACA/ACA-Annual/StPaul/StPaul.html

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